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Skeletal Muscle Glucocorticoid Receptor and Glutamine Synthetase Activity in the Wasting Syndrome in Rats Treated with 2,3,7,8-Tetrachlorodibenzo-p-Dioxin

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ABSTRACT

Skeletal Muscle Glucocorticoid Receptor and Glutamine Synthetase Activity in the Wasting Syndrome in Rats Treated With 2,3,7,8-Tetrachlorodibenzo-p-Dioxin. Max, S.R. and Silbergeld, E.K. (1986) Toxicol. Appl. Pharmacol. . This study demonstrated specific changes in rat skeletal muscles after a single oral dose (100 µg/kg) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The development of the wasting syndrome was characterized by marked body weight loss, as well as atrophy of plantaris and gastrocnemius muscles. Fourteen days after administration of TCDD, gastrocnemius muscle cytosolic receptor binding was significantly diminished while plantaris muscle glutamine synthetase activity was strikingly elevated, indicating that specific biochemical alterations occur in skeletal muscle in the wasting syndrome.

TCDD causes a striking wasting syndrome, characterized by weight loss in rats and other species (Seefeld et al., 1984a). Although the mechanism of the wasting syndrome remains undefined, it is temporally associated with decreased food consumption (Seefeld et al., 1984a). It has been hypothesized that changes in weight regulation and hypophagia account for TCDD-induced weight loss (Kelling et al., 1985; Rozman, 1984; Seefeld et al., 1984a,b). However, a number of morphological and metabolic changes differ from those observed in pair-fed control rats. These include alterations in lipid, carbohydrate and protein metabolism (Schiller et al., 1985; Christian et al., 1986b; Gasiewicz et al., 1980).

Skeletal muscle accounts for about 40% of rat body weight. Therefore, weight loss could reflect muscle wasting to a large degree. Yet, with the exception of the work of Christian et al. (1986b) skeletal muscle has been surprisingly neglected in studies of the wasting syndrome. Because of the likelihood that skeletal muscle is involved in the wasting syndrome, we measured muscle wet weight and protein content, as well as two biochemical parameters that are associated with muscle wasting: glucocorticoid receptor binding and glutamine synthetase activity. Elevated levels of glucocorticoid receptors have been noted in muscle atrophy resulting from a number of causes (Karpati, 1984 - review), including pharmacological doses of glucocorticoids (Konagaya et al., 1986). Muscle glutamine synthetase, which produces glutamine from amino acids derived from muscle proteins (Goldberg and Chang, 1978), increases in many stressful situations including starvation (Marliss et al., 1971), metabolic acidosis (Parry and Brosnan, 1978; King et al., 1983), and subsequent to in vivo (King et al., 1983) and in vitro (Smith et al., 1984; Max et al., 1986) glucocorticoid administration.

METHODS

Materials. Experiments were conducted according to a safety plan that involved the use of a biohazard containment facility for animals, protective equipment for personnel, and disposal according to EPA regulations. Rats (F344N, males, 200 g body weight, n = 13) were housed (3/cage) in plastic-bottomed cages in a glove box. They were given TCDD dissolved in acetone and suspended in sesame oil (p.o.) at a dose of 100 µg/kg in 0.1 ml/100 g body wt.; this high dose was selected because it is known to produce wasting and eventual death (Christian et al., 1986a,b). The rats were maintained in a 12-h lights on:off schedule. They were given Purina Lab Chow (#5001) and water ad libitum. Controls were treated with vehicle only (p.o.) at a volume equivalent to that given to the TCDD rats.

Experimental Protocols. Rats were weighed at the time of TCDD administration and at 7 and 14 days thereafter, when the experiment was terminated. They were sacrificed by an overdose of chloral hydrate. Plantaris and gastrocnemius muscles were removed and weighed for biochemical determinations.

Assays. Cytosolic glucocorticoid receptor binding was measured as described (Konagaya et al., 1986). Due to limited amounts of material, it was necessary to assess receptor binding at a single, saturating (15 nM) concentration of [^{3}H] triamcinolone acetonide. It is thus not possible to ascertain whether changes in receptor binding are a result of altered affinity or number of binding sites. Glutamine synthetase activity was assayed as described (Smith et al., 1984) using 5 mM glutamate [U-^{14}C] as substrate. Protein was determined by the method of Lowry et al. (1951).

Statistical Analysis. This was performed using Student's T-test.

RESULTS

Vehicle-treated, control rats gained weight (19%) during the 14 d course of the study. In contrast, TCDD-treated rats lost a striking 45% of their body weights during this period (Table 1). This is consistent with the findings of others in the wasting syndrome (Christian et al., 1986a; Seefeld et al., 1984a). To a large extent, loss of body weight reflects muscle weight loss (Table 1). The wet weight of plantaris muscles of TCDD-treated rats decreased by 45% compared with controls. However, when muscle wet weight is expressed as % body weight, muscles from TCDD-treated rats were significantly larger (18%) than from vehicle-treated animals. Gastrocnemius muscles from TCDD-treated rats lost weight to a similar extent (not shown).

Marked alterations in gastrocnemius muscle glucocorticoid cytosolic receptor binding and glutamine synthetase activity accompanied these changes. Receptor binding decreased to 43% of the control value 14 d following TCDD treatment (Table 2). Glutamine synthetase activity, on the other hand, increased 4.5-fold 14 d after TCDD treatment (Table 3).

DISCUSSION

We have shown striking abnormalities of rat skeletal muscle following a single high dose of TCDD. The severe muscle weight loss was expected since muscle constitutes 40% of the body mass of a mature rat. Therefore, loss of muscle , in addition to adipose tissue (Gasiewicz and Neal, 1979; Christian et al., 1986a; Kelling et al., 1985), might be responsible for the body weight loss in the wasting syndrome. This conclusion is supported by the data of Table 1, and it is suggested by the carcass protein measurements of Christian et al. (1986a). However, the present report appears to be the first in the TCDD wasting syndrome in which individual muscle weights are presented.

A significant decrease in cytosolic glucocorticoid receptor binding accompanied the muscle atrophy. This decrease was surprising in view of the work of DuBois and Almon (1980, 1981. They found an increase in muscle glucocorticoid receptor binding in a number of causes of muscle atrophy, including denervation and disuse (reviewed in Karpati et al., 1984). DuBois and Almon (1980, 1981) hypothesized that muscle atrophy, irrespective of cause, is accompanied by an increase in glucocorticoid receptor binding, and that such an increase may be etiologic in promoting atrophy. However, we have now shown an example of muscle atrophy in which this is not the case.

The reason for the decrease in glucocorticoid receptor binding and its sigificance in the wasting syndrome are not known. The decrease may be related to changes in circulating corticosteroid levels induced by TCDD (DeBartolomeis et al., 1984). Decreased receptor binding may reflect complex interactions between TCDD and glucocorticoid receptors. It is unlikely, however, that TCDD is acting directly via the glucocorticoid receptor, since receptor binding is decreased. Poellinger et al. (1985) have shown that, although they have many physical-chemical similarities, glucocorticoid and TCDD receptors are not identical; in vitro, TCDD does not compete for glucocorticoid receptor binding, and glucocorticoids do not compete with TCDD binding. On the other hand, TCDD and glucocorticoids do act synergistically in some situations, i.e., in causing cleft palates in mice (Birnbaum et al., 1986). Because of the well-known muscle atrophy caused by glucocorticoids (Konagaya et al., 1986), the role of glucocorticoids in this aspect of TCDD toxicity warrants further exploration.

The increase in glutamine synthetase activity in TCDD-treated rats is of interest in view of the work of Christian et al. (1986b), which demonstrated an increase in alanine and a decrease in glutamine in plasma from TCDD-treated

rats but not from pair-fed controls. Christian et al. (1986b) suggested that there was no increase in amino acid production from muscle in the wasting syndrome. Glutamine and alanine are selectively released from muscle, and are substrates for a number of tissues, notably intestine, liver, and kidney (Goldstein, 1986). Release of muscle alanine and glutamine appears to be regulated independently (Smith, 1986). Plasma and muscle glutamine declines to a greater extent than other amino acids during critical illness; the decline is related to the severity of the illness and to the degree of protein wasting (Askenazi et al., 1980; Goldstein, 1986). Starvation and metabolic acidosis increase the production and release of glutamine by muscle (Aikawa et al., 1973; Marliss et al., 1971). The extra glutamine is extracted by the kidneys and converted to glucose; the nitrogen is converted to ammonia (Goldstein, 1986; Parry et al., 1978). Possibly, glutamine was being extracted from the circulation at a high rate in the study of Christian et al., (1986b); their interesting data may be thus reconciled with those of the present study. The increase in glutamine synthetase activity after glucocorticoid administration (Max et al., 1986; Smith et al., 1984; King et al., 1983) and the selective changes in these amino acids noted above (Smith, 1986) suggest that enhanced amino acid mobilization from muscle protein may occur in the wasting syndrome.

We recently showed that glucocorticoids increase the level of glutamine synthetase mRNA in muscle cells in vitro (Max et al., 1986). We do not know whether the increase in enzyme activity caused by TCDD represents similar induction (Max et al., 1986). Nevertheless, TCDD causes changes in two biochemical parameters that have been associated with skeletal muscle wasting. The significance of these changes to the pathogenesis of the wasting syndrome remains to be explored.

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TABLE 1

BODY AND PLANTARIS MUSCLE WEIGHTS OF TCDD-TREATED RATS

Treatment	N	Body Weight		Plantaris Muscle	Wet Weight-Day 14
		Day 0	Day 14	(mg)	% Body Weight
Vehicle	6	210 ± 5[a]	250 ± 7[c]	226 ± 21	0.09 ± 0.01
TCDD	7	210 ± 7	115 ± 5[b,c]	123 ± 8[b]	0.11 ± 0.01[d]

Male Fisher 344 N rats were given 1 dose of TCDD (100 µg/kg p.o.). Rats were sacrificed and muscles removed 14 d after TCDD administration.

[a]Data are means ± SD

[b]Significantly different from vehicle-treated, $p < 0.0005$.

[c]Significantly different from day 0, $p < 0.005$.

[d]Significantly different from vehicle-treated, $p < 0.0025$.

TABLE 2

EFFECT OF TCDD ON RAT GASTROCNEMIUS MUSCLE CYTOSOLIC GLUCOCORTICOID RECEPTOR BINDING

Treatment	N	[^{3}H] Triamcinolone Acetonide Specific Binding (fmols/mg protein)
Vehicle	6	13.53 ± 3.82[a]
TCDD	7	5.83 ± 2.09[b]

[a]Data are means ● SD. Male Fisher 344 rats were given 1 dose of TCDD (100 µg/kg, p.o.). Rats were sacrificed and gastrocnemius muscles were removed 14 d after TCDD administration.

[b]Significantly different from vehicle, $p < 0.0005$.

TABLE 3

EFFECT OF TCDD ON GLUTAMINE SYNTHETASE ACTIVITY IN RAT PLANTARIS MUSCLES

Treatment	N	Glutamine Synthetase (nmols/h/mg protein)
Vehicle	6	5.23 ± 2.96[a]
TCDD	6	23.63 ± 11.47[b]

[a]Data are means ± SD. Male Fisher 344 rats were given 1 dose of TCDD (100 μg/kg, p.o.). Rats were sacrificed and plantaris muscles were removed 14 d after TCDD administration. Plantaris muscle weights are given in Table II.

[b]Significantly different from vehicle, $p < 0.025$.